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PGC-1/*spargel* counteracts high fat diet-induced obesity and cardiac lipotoxicity downstream of TOR and Brummer ATGL lipase

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SUMMARY

Obesity and metabolic syndrome are associated with an increased risk for lipotoxic cardiomyopathy, which is strongly correlated with excessive accumulation of lipids in the heart. Obesity- and type 2 diabetes-related disorders have been linked to altered expression of the transcriptional cofactor PGC-1α, which regulates the expression of genes involved in energy metabolism. Using *Drosophila*, we identify *PGC-1/spargel* (*PGC-1/srl*) as a key antagonist of high-fat diet (HFD)-induced lipotoxic cardiomyopathy. We find that HFD-induced lipid accumulation and cardiac dysfunction are mimicked by reduced *PGC-1/srl* function and reversed by *PGC-1/srl* overexpression. Moreover, HFD feeding lowers *PGC-1/srl* expression by elevating TOR signaling and inhibiting expression of the *Drosophila* adipocyte triglyceride lipase (ATGL, Brummer), both of which function as upstream modulators of *PGC-1/srl*. The lipogenic transcription factor SREBP also contributes to HFD-induced cardiac lipotoxicity, likely in parallel with *PGC-1/srl*. These results suggest a regulatory network of key metabolic genes that modulates lipotoxic heart dysfunction.

Keywords

heart; contractility; arrhythmia; cardiomyopathy; metabolic syndrome; type 2 diabetes; *brummer*; FAS; S6 Kinase; SREBP; epistasis; lipolysis; lipogenesis

INTRODUCTION

Heart failure is a major cause of mortality in modern society. The high prevalence of obesity and related diseases, including type 2 diabetes, plays a significant role in the increased incidence of cardiovascular disease, which affects more than a billion people worldwide. It is now recognized that obesity and diabetes are associated with lipotoxic cardiomyopathy, a

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form of cardiac dysfunction that is caused by excessive lipid accumulation in myocardial cells (Borradale and Schaffer, 2005; Christoffersen et al., 2003). The high incidence of obesity-associated heart dysfunction is aggravated by changes in nutritional habits, such as consumption of high caloric diets, combined with reduced physical activity. A major challenge in developing targeted therapeutics for this type of heart disease is to understand how the molecular and genetic changes induced by high caloric diets lead to metabolic imbalances that culminate in cardiac dysfunction.

Although mammalian model organisms are ultimately essential for identifying the underlying mechanisms of obesity and metabolic syndrome (Kanasaki and Koya, 2011; York and Bouchard, 2000). The genetic and metabolic complexity and gene redundancy in higher organisms complicates dissection of the fundamental genetic relationships involved in these diseases. However, the components and regulatory pathways of core metabolic pathways are generally highly conserved, making genetically simpler invertebrate models, such as *Drosophila*, well suited for such studies (Baker and Thummel, 2007; Botstein et al., 1997; Broughton et al., 2005; Diop and Bodmer, 2012; Kuehnlein, 2012; Noyes et al., 1995). *Drosophila* is unique in that it is the only invertebrate model organism with a beating heart for which suitable genetic tools and assays are available to study heart function (Bier and Bodmer, 2004; Ocorr et al., 2007; Wessells et al., 2004; Wolf et al., 2006; Ocorr et al., 2014).

Recent studies with *Drosophila* models of obesity-associated heart dysfunction have demonstrated that consumption of high-fat or -sugar diets, or genetic manipulation of key metabolic regulators, can lead to excessive fat accumulation accompanied by severe heart defects, including increased frequency of arrhythmias, reduced cardiac output, increased non-contractile myocardial cells, and altered myofibrillar structure and collagen content (Birse et al., 2010; Diop et al., 2012; Lee et al., 2010; Lim et al., 2011; Na et al., 2013). Thus, flies challenged with high caloric diets display metabolic responses and increased risk for heart disease reminiscent of those observed in humans. Importantly, these studies showed that inhibition of signaling through insulin, target of rapamycin (TOR) or the lipogenic transcription factor SREBP, or by increasing triacylglyceride (TAG) lipolysis were effective in counteracting excess lipid accumulation as well as the associated cardiac defects. Although these findings suggested that major metabolic pathways could be targeted for therapeutic intervention in human obesity-induced cardiomyopathy, further work is necessary to obtain a more complete understanding of the essential regulators of these lipogenic pathways and their relationship to cardiac function. In this study, we use the *Drosophila* model to delineate novel genetic relationships between key metabolic regulators relevant to cardiac lipotoxicity.

Peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1 (PGC-1) is a transcriptional coactivator first identified as a key regulator of thermogenesis in brown adipose tissue in mammals (Puigserver et al., 1998). Subsequently, additional PGC-1 family members have been shown to modulate gene expression by interacting with a number of nuclear receptors (Finck and Kelly, 2006; Gleyzer and Scarpulla, 2011; Lin et al., 2002). Studies in mice and flies have demonstrated that PGC-1 proteins are induced by environmental stimuli, such as cold exposure, exercise, or starvation, that trigger a number

of metabolic adaptations (Arany, 2008; Baar et al., 2002; Goto et al., 2000; LeMoine et al., 2008; Tiefenbock et al., 2010; Tinkerhess et al., 2012; Zechner et al., 2010). PGC-1α is the most studied member of the PGC-1 family and plays key roles in mitochondrial biogenesis and electron transport chain assembly by interacting with nuclear respiratory factor (NRF) and estrogen receptor-related receptor (ERR) transcription factors (Rowe et al., 2010; Russell et al., 2004; Scarpulla, 2011; Tiefenbock et al., 2010). PGC-1α also controls mitochondrial fatty acid oxidation in the muscles and liver by coactivating members of the PPAR and retinoic X receptor families of nuclear factors (Vega et al., 2000; Zechner et al., 2010). The PGC-1α/ERR axis has also been implicated in controlling cardiac metabolism and function (Finck and Kelly, 2006, 2007; Lai et al., 2008; Patten and Arany, 2012; Schilling and Kelly, 2011). Interestingly, decreased cardiac PGC-1α expression, observed in adipocyte triglyceride lipase (ATGL)-deficient mice, is associated with cardiac lipid accumulation and subsequent heart failure (Haemmerle et al., 2011). Of note, sequence variation at the PGC-1α locus has been correlated with obesity in humans (Rankinen et al., 2006). Despite the known involvement of PGC-1 in cardiac function, it is still unclear whether or how PGC-1 protein interacts with other metabolic regulators and signaling pathways in modulating the deleterious effects of high caloric diets on lipid accumulation and cardiomyopathy. In particular, it is not known if TOR and SREBP pathways interact with *PGC-1/srl* function in these processes.

In this study, we provide evidence that reducing the function of *Drosophila PGC-1/srl* causes a phenotype of excess fat accumulation and severe heart dysfunction that is remarkably similar to that observed in wildtype flies exposed to a HFD. This phenotype is exacerbated in *PGC-1/srl* mutant flies that are fed a HFD, whereas systemic or cardiac-specific *PGC-1/srl* overexpression is protective. Genetic interaction experiments suggest that *PGC-1/srl* is likely to act downstream of TOR signaling to protect the heart from lipotoxicity. We show that the protective effect of reduced TOR signaling requires elevated expression of *PGC-1/srl* and *ATGL* (major TAG lipase encoded by *brummer, bmm*, in *Drosophila*), with *ATGL/bmm* positioned downstream of TOR and upstream of *PGC-1/srl* in the homeostatic control of fat accumulation and cardiac lipotoxicity. Interestingly, these *PGC*-1/*srl* manipulations did not change the expression or processing of SREBP, and reducing SREBP does not significantly ameliorate heart dysfunction of *PGC-1/srl* heterozygotes, suggesting that *PGC*-1/*srl* and SREBP do not act in a strict epistatic relationship. Taken together, our data define a new interaction network of key metabolic regulators that contribute to cardiac lipotoxicity in *Drosophila*, and thus provide insights into possible mechanisms of HFD-associated cardiomyopathy relevant to humans.

RESULTS

HFD-Induced Fat Accumulation Requires Reduced *PGC-1/srl* Expression

To determine the involvement of *Drosophila PGC-1/srl* in obesity and heart dysfunction, we first examined the effect of *PGC-1/srl* modulation on whole body triglyceride (TAG) content in wildtype (w^{1118}) flies, heterozygous *PGC-1/srl* mutants ($PGC\text{-}1^{XP/+}$ and $PGC\text{-}1^{PBAC/+}$), and flies with systemic RNAi-mediated *PGC-1/srl* knockdown (KD). Flies were raised on normal food (NF) for 5–10 days and then transferred for a further 5 days to NF or HFD (NF

supplemented with 30% coconut oil). We found that flies with reduced *PGC-1/srl* function had elevated TAG levels compared with wildtype flies, and TAG levels were further increased when the mutant or systemic *PGC-1/srl* KD flies (Arm>*PGC-1*^{RNAi}) were fed a HFD (Figures 1A, 1B, S1A). Moreover, TAG levels were increased in the heart and muscle-rich thorax of HFD-fed or *PGC-1/srl* heterozygous flies (Figures 1C and S1B), consistent with the deposition of TAG in additional organs once the capacity of adipose tissue was exceeded. To further demonstrate that the increased TAG level in *PGC-1/srl* heterozygous mutant flies was specific to reduced PGC-1/srl function, we used a genomic rescue construct (*PGC-1*^{GR}; Tiefenbock et al., 2010) in flies carrying the *PGC-1*^{XP} mutation. We found that these flies (*PGC-1*^{XP}/*PGC-1*^{GR}) have a significantly lower TAG level compared to *PGC-1* heterozygous flies (*PGC-1*^{XP}/+) under NF or HFD (Figure 1A). These experiments show that a genomic *PGC-1/srl* transgene was able to rescue the TAG phenotype of the *PGC-1/srl* mutant flies in support of the idea that the observed phenotype is specific to loss of *PGC-1/srl* function. The rescue flies show a somewhat lower TAG level than the wildtype control, which possibly reflects a slightly higher activity by the rescue transgene compared to the wildtype allele. *PGC-1/srl* KD specifically in adipose tissue (using the *lsp*-Gal4 driver) or muscle (24B-Gal4 driver) also resulted in systemic fat accumulation in both NF- and HFD-fed flies, supporting an association between reduced *PGC-1/srl* function and fat accumulation (Figure S1C and S1I).

Because wildtype flies on a HFD and flies with reduced *PGC-1/srl* function on NF had similar levels of TAG, we asked whether *PGC-1/srl* expression was modulated by HFD feeding. Indeed, qPCR analysis showed that HFD decreased *PGC-1/srl* mRNA in various tissues to a level comparable to PGC-1/*srl* heterozygotes, including in the abdomen, the heart and muscle-rich thorax (Figures 1D-1F, S1D and S1H). Conversely, TAG levels were decreased in HFD-fed flies overexpressing *PGC-1/srl* systemically (Figures 1G, S1G and S1J) or specifically in muscle (Figure S1E) or adipose tissue (Figure S1F). These findings suggest that the HFD-induced reduction in *PGC-1/srl* function promotes fat accumulation, whereas overexpression of *PGC-1/srl* blunts the effect.

To further analyze the effects of HFD and reduced *PGC-1/srl* function on lipid accumulation, we visualized lipid droplets in cardiomyocytes by Nile Red staining. We observed an increase in droplet size and fat content (Figure 1H) in the hearts of wildtype flies on HFD *and PGC-1/srl* heterozygotes on NF or HFD.

HFD-Induced Reduction in *PGC-1/srl* Expression Provokes Cardiac Lipotoxicity

The finding that *PGC-1/srl* heterozygotes and HFD-fed wildtype flies accumulated fat in the heart (Figures 1C and 1H), together with the reported link between obesity and heart disease in humans and other mammals (Borradaile and Schaffer, 2005; Zhou et al., 2000), prompted us to investigate the effect of *PGC-1/srl* manipulation on heart function in *Drosophila*. For this, we used the previously established methods of semi-intact heart preparation, high-speed video recording, and SOHA software analysis (Ocorr et al., 2007; Fink et al., 2009). We observed that wildtype flies on HFD and flies with reduced *PGC-1/srl* function on either NF or a HFD showed decreased heart period (Figure 2A, 2B and 2D and Movies S1–S4) due to reductions in both diastolic and systolic intervals (Figure S2A, S2B, S2D, and S2E). This is

also illustrated in M-mode traces (Figures 2D and 2E). Conversely, systemic *PGC-1/srl* overexpression increased the heart period even under HFD conditions (*PGC-1*^{EP} flies; Figures 2C and 2E), which was primarily due to an increased diastolic interval (Figure S2C and S2F). Wildtype flies on HFD also showed reduced fractional shortening (Figure S2G and S2H), as previously noted (Birse et al., 2010), but this parameter was unaffected by reduced *PGC-1/srl* function (Figure S2G and S2H).

PGC-1/srl heterozygotes exhibited striking defects in other aspects of cardiac contraction that were remarkably similar to those observed upon HFD feeding. The defects included heart tubes with regions lacking active contractions ('non-contractile'), asynchronous anterior versus posterior beating patterns ('asynchronous beating'), dysfunctional inflow valves (ostia), and occasional localized constriction ('other defects') (Movies S5–S7). Quantification of these parameters revealed that cardiac dysfunction was markedly increased in wildtype flies fed a HFD and in flies with reduced *PGC-1/srl* function (*PGC-1*$^{xp/+}$), and was further increased in *PGC-1*$^{xp/+}$ flies on a HFD (Figures 2F and 2G). Remarkably, *PGC-1/srl* heterozygous flies containing a genomic *PGC-1/srl transgene* (*PGC-1*^{XP}/*PGC-1*^{GR}) showed a significant decreased in cardiac dysfunction under NF and HFD (Figures 2F and 2G), suggesting that adding a genomic copy of *PGC-1/srl* to *PGC-1/srl* heterozygotes rescued also the heart phenotypes. These data therefore suggested that the HFD-induced reduction in *PGC-1/srl* function is associated not only with fat accumulation but also with HFD-induced heart dysfunction.

To determine whether cardiac-specific KD of *PGC-1/srl* was sufficient to cause heart dysfunction, we expressed two different *PGC-1/srl* RNAi constructs using a cardiomyocyte-specific driver (GMH5). Indeed, flies with *PGC-1/srl* KD exhibited pronounced hearts defects comparable to those observed in *PGC-1/srl* heterozygotes or wildtype flies fed a HFD (Figures 2F and 2G), indicating that reducing *PGC-1/srl* function in the heart alone was sufficient to cause dysfunction. The heart defects tended to be more severe when cardiac *PGC-1/srl* KD flies were exposed to a HFD, whereas HFD-fed flies with cardiac overexpression of *PGC-1/srl* (*PGC-1*^{EP}) showed a much lower incidence of heart defects (Figures 2F and 2G). These data suggest that high levels of *PGC-1/srl* protected the heart from HFD-induced dysfunction in a cardiac-autonomous fashion. Conversely, downregulation of *PGC-1/srl* activity correlated with the deleterious effects of HFD on cardiac function, although it cannot be ruled out that reduced *PGC-1/srl* function also has effects on the heart not related to fat accumulation.

Inhibition of TOR Activity Elevates *PGC-1/srl* Expression and Protects Against HFD-Induced Heart Dysfunction

We previously showed that reduced TOR signaling can protect against HFD-induced obesity and heart dysfunction in *Drosophila* (Birse et al., 2010). Here, we find that HFD feeding of wildtype flies activated TOR signaling, as shown by increased levels of the phosphorylated (i.e. active) forms of AKT (P-AKTS505) and S6K (P-S6KT398) (Figure 3A–C). These observations are consistent with the previously demonstrated protective effect of reduced TOR activity in lipotoxic heart dysfunction (Birse et al., 2010).To determine whether *PGC-1/srl* and *TOR* might function through a common pathway, we examined activation of

AKT (upstream regulator of TOR) and S6K (downstream effector of TOR) in *PGC-1/srl* heterozygotes. Like wildtype flies fed HFD, PGC-1 heterozygotes flies show an increase of P-AKTS505 and P-S6KT398 under HFD, however, P-AKT and P-S6K levels did not appear to be altered differently in *PGC-1/srl* heterozygous flies compared to wildtype (Figure 3A–C), suggesting that *PGC-1/srl* was unlikely an upstream regulator of TOR signaling in this situation.

We next asked whether manipulation of TOR signaling affected *PGC-1/srl* expression. Indeed, NF-fed flies with reduced TOR signaling ($TOR^{7/P}$, previously shown to protect from HFD-induced heart dysfunction; Birse et al., 2010) had significantly increased *PGC-1/srl* mRNA levels (Figure 3D), as did flies with systemic expression of dominant-negative S6K ($S6K^{DN}$; Figure S3B). Remarkably, the HFD-induced reduction in *PGC-1/srl* mRNA level was reversed in $TOR^{7/P}$ mutants (Figure 3D). These data therefore suggested that reducing *PGC-1/srl* expression in $TOR^{7/P}$ mutants might reduce or abrogate the protective effect of reduced TOR signaling on HFD-induced fat accumulation. To test this, we created flies with reduced function of both *TOR* and *PGC-1/srl* ($TOR^{7/P};PGC\text{-}1^{xp/+}$). In keeping with our hypothesis, the double-mutant flies had elevated levels of TAG, similar to those in $PGC\text{-}1^{XP/+}$ flies, and TAG levels were further increased upon HFD feeding (Figure 3F).

Next, we tested whether *PGC-1/srl* may also function downstream of *TOR* in regards to heart function. Indeed, we found a high incidence of heart dysfunction in $TOR^{7/P};PGC\text{-}1^{xp/+}$ double-mutant flies, similar to that observed for single $PGC\text{-}1^{XP/+}$ mutants (Figure 3G and 3H). These data suggested an epistatic relationship between *PGC-1/srl* and *TOR*, in which TOR acted as a negative upstream regulator of *PGC-1/srl* function in controlling fat content and heart function (Figure 3I). The findings further suggested that the previously demonstrated cardioprotective effect of reduced TOR signaling may require upregulation of *PGC-1/srl* expression.

The ATGL/Bmm Lipase Acts Downstream of TOR and Upstream of *PGC-1/srl* in Modulating HFD-Induced Heart Dysfunction

Lipolysis, which is the process by which triglycerides are degraded into free fatty acids, is a major component of lipid metabolism. Brummer (*bmm*), which encodes the *Drosophila* homologue of mammalian ATGL lipase, also mediates fat hydrolysis in this invertebrate model system (Gronke et al., 2005). We previously showed that *bmm* overexpression efficiently prevented HFD-induced fat accumulation and heart dysfunction (Birse et al., 2010). Therefore, we asked how Bmm might contribute to the TOR- and PGC-1-regulated effects on lipid accumulation and cardiac function. Interestingly, the phenotype of *bmm* heterozygous flies was remarkably similar to that of *PGC-1/srl* heterozygotes. Thus, $bmm^{1/+}$ flies accumulated excess fat and considerable heart dysfunction on NF, which were further increased by HFD feeding (Figures 3F–H). Conversely, systemic *bmm* overexpression prevented HFD-induced TAG accumulation (Figure S3A) and heart dysfunction (Birse et al., 2010). Importantly, *bmm* heterozygotes also exhibited heart dysfunction that was further aggravated by HFD (Figures 3G and 3H). These findings raised the possibility that ATGL/*bmm* acts in the same pathway as *PGC-1/srl* and *TOR* in modulating cardiac lipotoxicity.

To test this idea, we first examined *bmm* expression in $TOR^{7/P}$ mutant flies. As was observed for *PGC-1/srl* expression (Figure 3D and S3B), *bmm* mRNA levels were increased in $TOR^{7/P}$ and $S6K^{DN}$ flies (Figure 3E and S3C). Conversely, *bmm* mRNA was reduced by HFD feeding, and this was reversed in $TOR^{7/P}$ mutant flies (Figure 3E), suggesting that *bmm* acts downstream of TOR signaling and may be required for the fat-lowering effect of reduced TOR function. To examine this possibility, we created double-mutant flies with reduced *TOR* and *bmm* function ($TOR^{7/P};bmm^{1}/+$). Compared to the protective effect of $TOR^{7/P}$ from HFD effects, $TOR^{7/P};bmm^{1}/+$ and $bmm^{1}/+$ mutant flies exhibited similarly increased TAG level and severe degrees of heart dysfunction on both NF and HFD, as wildtype flies on HFD (Figures 3F–H), consistent with *bmm* loss of function compromising the protection conferred by reduced TOR signaling. These data therefore suggest that like with *PGC-1/srl*, TOR pathway activation under HFD leads to reduced *bmm* expression, which in turn promotes fat accumulation and heart dysfunction (Figure 3I).

Next, we wanted to test whether adult-only manipulation of *bmm* or *PGC-1/srl* was sufficient to mimic (or protect from) HFD-induced fat accumulation and heart dysfunction. For this purpose, we used the Gene Switch system (GS; Osterwalder et al., 2001; Roman et al., 2001) to conditionally drive - upon RU-486 addition - gene expression in the adult heart, both myocardial and pericardial cells (Hand-GS-Gal4; Monnier et al., 2012). Administration of ethanol vehicle or RU-486 to wildtype flies did not noticeably affect ATGL/*bmm* or PGC-1/*srl* expression, fat content or heart function (Figure S3G–K). However, cardiac KD of ATGL/*bmm* (*Hand-GS>*bmm^{RNAi}) by RU treatment caused an increase in systemic fat content compared to ethanol vehicle exposure; and conversely, conditional ATGL/*bmm* or PGC-1/*srl* overexpression in the adult heart (*Hand-GS>UASbmm* or *Hand-GS>*$PGC\text{-}1^{JW}$) decreased fat levels (Figure S3D), suggesting metabolic gene manipulation in cardiac tissue can have a systemic effect (see Grueter CE et al., 2012). Importantly, adult-specific overexpression of PGC-1/*srl* or ATGL/*bmm* in the heart of flies treated with RU (*Hand-GS>*$PGC\text{-}1^{JW}$, *Hand-GS>bmm*) showed markedly decreased heart dysfunction under HFD, compared to ethanol controls (Figure S3E and S3F). In contrast, *Hand-GS>*$PGC\text{-}1^{RNAi}$ flies treated with RU exhibited significantly increased heart dysfunction under NF and HFD, compared to ethanol controls (Figure S3E and S3F). These data demonstrate that PGC-*1/srl and* ATGL/*bmm* manipulation during adult stages is sufficient to modulate heart function under NF and HFD. Together with HFD feeding of adult flies causing increased TOR signaling (Figure 3A) and decreased *PGC-1/srl* and ATGL/*bmm* expression (Figure 3D and 3E), these data strongly suggest that *PGC-1/srl* and ATGL/*bmm* act as mediators of HFD induced heart dysfunction and effectors of TOR signaling.

To investigate possible genetic interactions between *PGC-1/srl* and *bmm*, we first analyzed *PGC-1/srl* mRNA levels in *bmm* mutant flies. We found that *PGC-1/srl* RNA levels were significantly increased in flies with systemic overexpression of *bmm* and significantly reduced in *bmm* heterozygotes (Figures 4A – D). Using the Gene Switch system, we found that induced adult heart expression or KD of ATGL/*bmm* (Hand-GS>*UASbmm-RU* or Hand-GS>Bmm^{RNAi}) leads to increased or decreased PGC-1/*srl* expression in the heart, respectively (Figure S4A–D), which is consistent with PGC-1/*srl* acting downstream of ATGL/*bmm* in causing (counteracting) HFD-induced heart dysfunction. In *PGC-1/srl* heterozygous flies on the other hand, *bmm* mRNA levels were similar to wildtype (Figure

4E–F). Moreover, heart-only induced expression of PGC-1/*srl* with *Hand-GS* also did not significantly alter ATGL/*bmm* expression (Figure S4E–F). Interestingly, however, hand-GS induced cardiac *PGC-1/srl* KD did exhibit decreased ATGL/*bmm* levels (Figure S4G–H), suggesting that there may be some feedback regulation from PGC-1/*srl* to ATGL/*bmm*, although ATGL/*bmm* acts predominantly upstream *PGC-1/srl* in modulating HFD-related cardiac dysfunction (see below and Figures 4G–H and S4I–J).

We next examined the relationship between *PGC-1/srl* and *ATGL/bmm* in regulating heart function. For this purpose, we examined *bmm* heterozygous flies with cardiac *PGC-1/srl* overexpression (*Hand>PGC-1*^{JW}*;bmm*^{1}*/+*). We found that cardiac *PGC-1/srl* overexpression was able to rescue the heart dysfunction phenotype of *bmm* heterozygotes under NF and HFD, as well as a control rescue with *ATGL/bmm* overexpression (Figure 4G and 4H). As expected, we also found that cardiac-specific KD of *ATGL/bmm* using the myocardial-specific Tin-HE-Gal4 driver induced a high proportion of heart defects in both NF- and HFD-fed flies (Figure S4I–J). However, concomitant cardiac overexpression of *PGC-1/srl* significantly reduced the deleterious effects of *ATGL/bmm* KD (Figure S4I–J). Taken together, these data are consistent with *ATGL/bmm* acting upstream of *PGC-1/srl* in regulating heart function. These findings suggest that HFD-induced lipid accumulation and cardiac dysfunction are accompanied by the sequential activation of TOR, downregulation of *ATGL/bmm* and subsequently *PGC-1/srl* expression, thus leading to cardiac lipotoxicity (Figure 4I).

PGC-1/srl and *SREBP* Likely Act in Parallel to Modulate HFD-Induced Heart Dysfunction

SREBP is a conserved transcriptional regulator of lipogenic gene expression (Dobrosotskaya et al., 2002; Osborne and Espenshade, 2009; Seegmiller et al., 2002). Activation of SREBP or its lipogenic targets, including fatty acid synthase (FAS), increases fat accumulation and causes heart dysfunction in *Drosophila* (Lim et al., 2011), similar to the effects of reduced *PGC-1/srl* function (Figures 1 and Figure 2). To determine the effect of HFD on processing of full-length (FL) SREBP to the transcriptionally active mature form (m-SREBP), we created flies carrying a sensor for m-SREBP–mediated transcriptional activation (Kunte et al., 2006) together with a UAS-nGFP reporter. In these flies, activation of m-SREBP is indicated by an increase in GFP fluorescence. We observed that flies fed a HFD showed higher levels of nuclear GFP in the heart (Figure 5A) and thoracic muscle (Figure S5A) than NF-fed flies, indicating that SREBP activity was induced by the HFD. In keeping with this, expression of the SREBP target gene *FAS* was also elevated in HFD-fed compared to NF-fed flies (Figure 5E).

Interestingly, FAS expression was also increased in *PGC-1/srl* heterozygotes compared to wildtype flies on both NF and HFD (Figures 5E). Also, *bmm* overexpression and mutant flies have decreased and increased FAS levels, respectively (Figure S5B and S5C). Therefore, we asked whether SREBP and *PGC-1/srl* interacted to induce fat accumulation and heart dysfunction in response to the HFD. For this purpose, processing of FL-SREBP to m-SREBP was analyzed by western blot analysis. Wildtype flies fed a HFD showed increased levels of both FL-SREBP and m-SREBP compared with flies on NF (Figure 5B–D); however, SREBP expression in *PGC-1/srl* heterozygotes, which contain similar levels of

TAG on NF as do wildtype flies on HFD, was not significantly different from wildtype flies in either NF or HFD conditions (Figure 5B–D). Similarly, SREBP heterozygotes expressed reduced levels of *FAS* mRNA (Figure 5F), but not of *PGC-1/srl* mRNA (Figure 5G). Therefore, these data suggest that *PGC-1/srl* and *SREBP* do not affect each other's expression in their modulation of fat accumulation, and may occur through distinct mechanisms.

Finally, we examined directly the relationship between *SREBP* and *PGC-1/srl* in modulating heart function. Cardiac dysfunction was slightly less prevalent in HFD-fed *SREBP* heterozygotes than in wildtype flies (Figure 5H and 5I), suggesting that SREBP partially mediate the deleterious effect of HFD on the heart., *SREBP* and *PGC-1/srl* double-heterozygous flies exposed to a HFD showed a heart phenotype strikingly similar to that of *SREBP* heterozygotes (Figures 5H and 5I). In addition, we found that *PGC-1/srl and FAS* double KD in the heart produced only a slight reduction of heart dysfunction compared to *PGC-1/srl* single KD (Figure S5D–E), which would be consistent with SREBP being the dominant regulator of FAS in the heart (see Lim et al., 2011) and *PGC-1/srl* also acting through other effectors. Overall, the data suggest that *SREBP* may at least in part act in parallel to *PGC-1/srl*, but it cannot be ruled out that *PGC-1/srl* also acts downstream (or *vice versa*), through FAS and possibly other effectors, in regulating heart function (illustrated in Fig. 6). Collectively, these findings are consistent with a model in which SREBP and Bmm-PGC-1/Srl function downstream of TOR and in parallel to each other, interconnected via FAS, to control fat accumulation and heart function. In conclusion, we found that HFD causes excess fat accumulation and cardiac dysfunction via TOR-dependent activation of two parallel pathways, TOR–ATGL/Bmm–PGC-1/Srl and TOR–SREBP (Figure 6).

DISCUSSION

It is well established that lipid accumulation in cardiomyocytes can lead to cardiac dysfunction (e.g. Zhang and Ren, 2011), but the genetic and metabolic regulatory networks that mediate cardiac lipotoxicity have yet to be fully understood. Here, we used the *Drosophila* model to investigate the genetic relationships between *PGC-1/srl* and other metabolic regulators to elucidate the mechanisms of HFD-induced obesity and cardiac dysfunction. We found that reducing *PGC-1/srl* function was sufficient to induce TAG accumulation and cardiac lipotoxicity, a phenotype remarkably similar to that observed in wildtype HFD-fed flies. Indeed, HFD feeding reduced *PGC-1/srl* expression wildtype flies. Conversely, *PGC-1/srl* overexpression countered both the fat accumulation and associated heart defects induced by HFD, indicating that *PGC-1/srl* is both necessary and sufficient for counteracting obesity and associated cardiac lipotoxicity. In humans, genome-wide association studies conducted with various ethnic and geographical cohorts have suggested a possible link between obesity and variants in *PGC-1* genes (Muller el al., 2003; Ayra et al., 2004). Moreover, reduced *PGC-1* gene expression was observed in patients with diabetes and those developing insulin resistance, but no clear mechanism of action has been established (Mootha et al., 2003; Patti et al., 2003). In mammals, *PGC-1* genes are known to control cardiac metabolism and function (Schilling and Kelly, 2011; Lai et al., 2008; Russel et al., 2004; Rowe et al., 2010), and reduced PGC-1α has been linked to heart failure and hypertrophy in rodent models of heart disease (Lehman and Kelly, 2002; Arany et al., 2006;

Huss et al., 2006). Thus, our demonstration of a close association between *PGC-1/srl* function, excess fat accumulation, and cardiac lipotoxicity further supports a critical role for this coactivator in diabetic cardiomyopathy. In addition, we demonstrate for the first time that reduction *PGC-1/srl* function is essential in mediating HFD-induced heart dysfunction.

We demonstrated that HFD feeding activates TOR signaling (increased P-AKT and P-S6K), which in turn leads to downregulation of *PGC-1/srl* expression. In contrast, a reduction in TOR function, which protects against obesity and heart dysfunction, is associated with increased *PGC-1/srl* levels. Furthermore, reducing both PGC-1*/srl* and *TOR* function (*$TOR^{7/P}$;$PGC\text{-}1^{xp/+}$*) abrogated the protective effects of TOR reduction against HFD-induced obesity and heart dysfunction. These findings therefore suggest that *PGC-1/srl* is a negative downstream effector of TOR. We suggest that persistent activation of TOR in response to a HFD reduces *PGC-1/srl* expression, which in turn leads to lipid accumulation in the myocardium and provokes heart dysfunction.

We also identified ATGL/Bmm lipase as a downstream effector of TOR in HFD-induced obesity and heart dysfunction, and the effects of a HFD were similar in *bmm and PGC-1/srl* mutant flies. Importantly, *bmm* RNA levels were increased in TOR mutant flies and – as observed with *PGC-1/srl* – reduction of *bmm* function in TOR mutant flies (*$TOR^{7/P}$;bmm/+*) eliminated the beneficial effects of TOR. Because modulation of *bmm* dominantly induced changes in *PGC-1/srl* mRNA levels and cardiac-specific increase in *PGC-1/srl* levels in *bmm* KD flies protected against heart dysfunction, we conclude that *PGC-1/srl* acts downstream of *bmm* and TOR, thus delineating a genetic pathway from *TOR* to *PGC-1/srl* via *ATGL/bmm*.

Interestingly, a recent study showed that mammalian ATGL controls murine heart dysfunction via PPARs (Haemmerle et al., 2011). Here, we demonstrated that this relationship also appears to exist in the fly, and importantly, provides strong evidence that under HFD conditions *ATGL/bmm* and *PGC-1/srl* act downstream of TOR in mediating cardiac lipotoxicity. Interestingly, in the above study cardiac *PGC-1* gene expression is also reported to be reduced with ATGL deficiency (Haemmerle et al., 2011), thus placing *PGC-1* gene function downstream of ATGL in both the fly and the mouse heart. Although molecules of equivalent function to mammalian PPARs have not yet been conclusively identified in *Drosophila*, our findings are consistent with the presence of a fundamentally conserved epistatic relationship between *TOR*, *bmm*, and *PGC-1/srl* in the metabolic control of cardiac lipotoxicity.

Previous studies have suggested that TOR stimulates lipogenesis via a SREBP-dependent increase in *FAS* expression (Porstmann et al., 2008; Hagiwara et al., 2012), and we have recently shown that SREBP lipogenic activity strongly influences heart function in *Drosophila* (Lim et al., 2011). In this study, we demonstrate that under HFD conditions, *PGC-1/Srl* and SREBP do not seem to directly regulate each other and may act independently to regulate fat accumulation and heart function, which implies that HFD activation of TOR signaling triggers parallel but partially interdependent pathways to regulate lipogenesis (through FAS). This does not preclude that *PGC-1/srl* and *SREBP* (as well as *bmm*) also have FAS-unrelated functions in modulating fat accumulation and cardiac

function. In our model, we propose that HFD activation of the nutrient-sensing TOR pathway leads to downregulation of the *bmm–PGC-1/srl* axis and to upregulation of the *SREBP* axis, both resulting, at least in part, in FAS upregulation. Such a mechanism would have two deleterious consequences: increased circulating fatty acid levels and increased storage of fat in non-adipose tissues, leading to lipotoxicity and cardiac dysfunction. Interestingly, rodent models of obesity also show increased TAG deposition in the heart and subsequent development of cardiac lipotoxicity (Borradale and Schaffer, 2005; Zhou et al., 1989; Christoffersen et al., 2003). These findings suggest that HFD feeding perturbs homeostatic pathways that are tightly regulated when fat intake is normal.

In humans, type-2 diabetes and associated diseases of the metabolic syndrome are aggravated with age. A recent study in *Drosophila* by Rera et al. (2011) have shown that *PGC-1/srl* expression decreases with age in the intestine and *PGC-1/srl* overexpression was sufficient to delay tissue aging, at least in part by increasing mitochondrial activity. These data are consistent with our findings that *PGC-1/srl* overexpression protects against obesity and cardiac dysfunction. Further studies will be required to determine our proposed network of gene regulation is also operational in regards to *PGC-1/srl*'s role in cardiac and overall aging. Conversely, it will be of interest to determine whether increasing certain aspects of mitochondrial function in the heart is sufficient to counteract the cardiac lipotoxicity of HFD feeding.

In a recent report TOR signaling was found to function as a positive regulator of PGC-1/*srl* in growth process during larval development of *Drosophila* (Mukherjee and Duttaroy, 2013), which is consistent with previous findings that PGC-1/*srl*-deficient *Drosophila* larvae exhibit dramatic growth impairment (Tiefenbock et al., 2010). In contrast, however, we provided strong evidence in this study that during metabolic homeostasis in the adult animal, PGC-1/*srl* is a negative effector of HFD-induced augmentation of TOR signaling. Thus, during organismal and tissue growth, PGC-1/*srl* mediates TOR signaling. But in the adult upon a metabolic or dietary insult, activation of TOR function and downstream ATGL/*bmm* lipase inhibition, prevents PGC-1/*srl* function, establishing a new genetic relationship between TOR and PGC-1/*srl*.

In this study, we have identified an integrated genetic network of metabolic regulators in the control cardiac lipotoxicity, in particular, PGC-1/*srl* acting as a negative effector of TOR. The network connects insulin–TOR, ATGL/Bmm, PGC-1/Srl, SREBP, and FAS, all of which have been associated with obesity in humans (Rankinen et al., 2006; Yang et al., 2007), but their relationships has not been comprehensively elucidated. Our work thus identifies a prototypical gene network with significant relevance for understanding lipotoxic cardiomyopathy in humans, and further emphasizes the utility of the *Drosophila* heart model in unraveling complex metabolic processes in higher organisms.

EXPERIMENTAL PROCEDURES

HFD Feeding Regimen

Flies were aged for 5 days after eclosion in tubes (25 flies/tube) containing normal fly food (NF) and then transferred to NF or HFD (NF + 30% coconut oil) for 5 days, as previously

described (Birse et al., 2010). Identical groups of flies were either weighed and frozen at −80°C for determination of TAG content (Birse et al., 2010) or analyzed for heart function abnormalities (see below).

Semi-Intact *Drosophila* Heart Preparation and Digital High-Speed Movie Analysis

All dissection steps were performed in artificial hemolymph saline, as previously described (Ocorr et al., 2007). Flies were anesthetized with fly-nap (Carolina Biochem.) and transferred to a Vaseline-coated Petri dish for dissection as described (Vogler and Ocorr, 2009). The submerged dissected hearts were oxygenated for 15 min at room temperature to equilibrate. High-speed digital movies were made using a Leica DM-LFSA microscope with a 10× water immersion lens, a Hamamatsu EM-CCD camera, and HCI image capture software (Hamamatsu, Inc.). Movies were analyzed with custom designed software (Fink et al., 2009; Ocorr et al., 2007). See also supplemental experimental procedures and references.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- *PGC-1/srl* mutations mimic HFD-induced cardiac lipoxicity
- HFD-induced cardiac lipotoxicity requires *PGC-1/srl* inhibition
- The effect of TOR on *PGC-1/srl* is mediated by ATGL/*bmm* inhibition
- *PGC-1/srl* and SREBP act via parallel pathways to control cardiac lipotoxicity

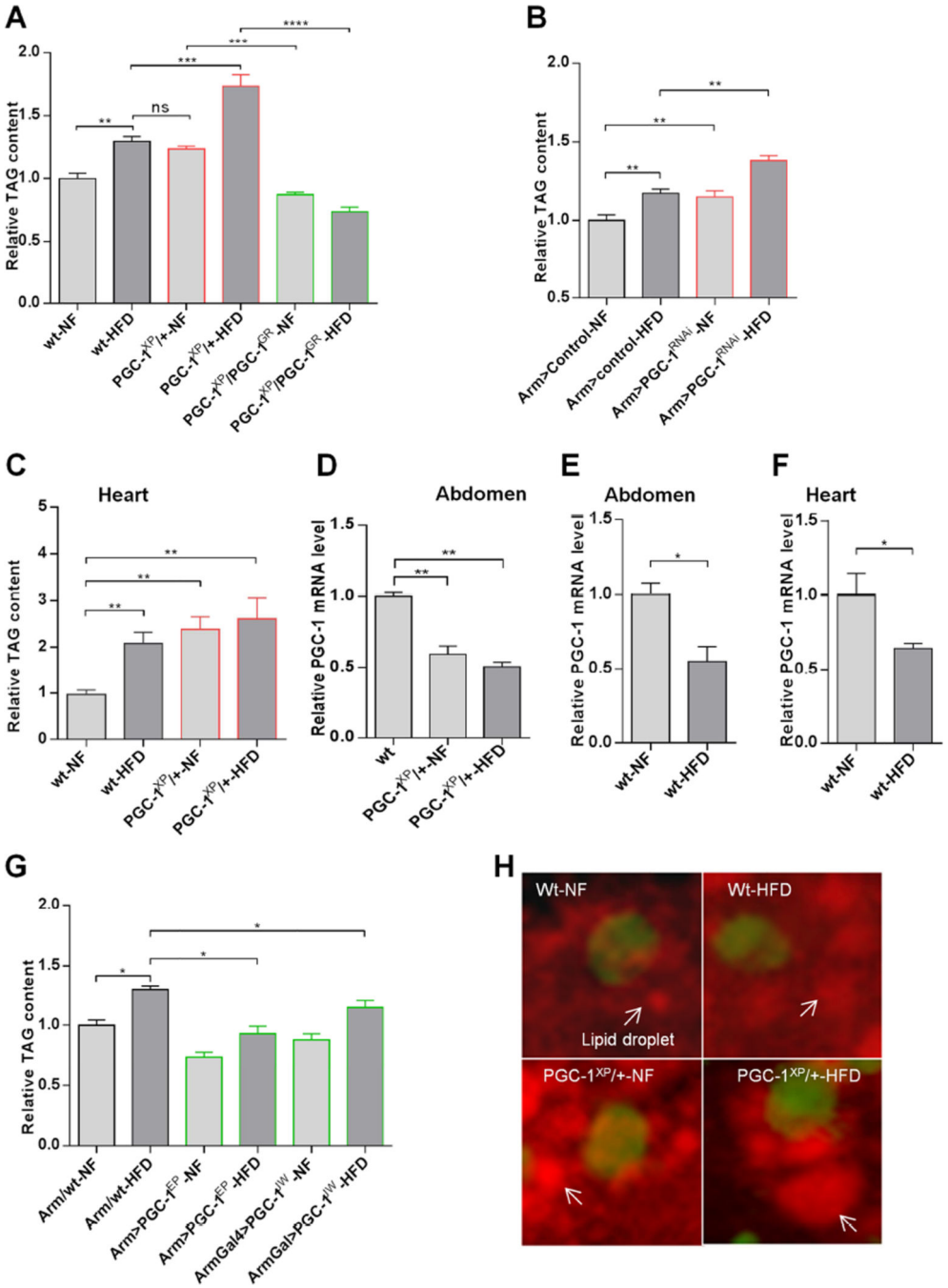

Figure 1. HFD-Induced Obesity Is Accompanied by a Reduction in *PGC-1/srl* Function

(A) TAG content of *PGC-1/srl* mutant flies. Flies were fed for 5–10 days on normal food (NF) and then transferred to NF or HFD (NF + 30% coconut oil) for a further 5 days. The wildtype (wt) strain is w^{1118}. $PGC\text{-}1^{XP}$/+and $PGC\text{-}1^{GR}$ are respectively *PGC-1* heterozygous mutant and and PGC-1GR denotes flies that carry a wild type PGC-1 genomic rescue transgene. Results are expressed as the fold difference in whole fly TAG compared with wt-NF flies and are the mean ± SEM of n = at least 27 for all genotypes and conditions

(wt-NF replicates were normalized to their average, thus contain error bars here and throughout). **P < 0.01, ***P < 0.001, ****P <0. 0001,one way ANOVA test.

(B) TAG content of *PGC-1* knockdown flies. Controls (GD control lines used to make PGC-1 RNAi) are the same genetic background as the $PGC\text{-}1^{RNAi}$ line. Results are expressed as the fold difference in whole fly TAG compared with Arm>control-NF flies and are the mean ± SEM of $42 \leq n \geq 27$. **P < 0.01, one way ANOVA test.

(C) TAG content in the hearts of *PGC-1/srl* mutant flies. Results are expressed as the fold difference compared with wt-NF flies and are the mean ± SEM of $250 \leq n \geq 200$. . **P < 0.01, one way ANOVA test.

(D–F) Relative *PGC-1* mRNA levels in wt or *PGC-1/srl* mutant flies. *PGC-1* mRNA was measured by qPCR in (D) the abdomen of wt and $PGC\text{-}1^{XP/+}$ heterozygous flies, and (E) the abdomen or (F) the heart of wt flies on NF or HFD. Results are expressed as the fold difference compared with wt flies and are the mean ± SEM of triplicates. *P < 0.05, **P < 0.01, ANOVA and student t-tests.

(G) TAG content of flies overexpressing *PGC-1/srl*. Results are expressed as the fold difference in whole fly TAG compared with Arm/wt-NF flies and are the mean ± SEM of $36 \leq n \geq 27$. . *P < 0.05, one way ANOVA test.

(H) Representative images of Nile Red staining of lipid droplets in cardiomyocytes of wt and $PGC\text{-}1^{XP/+}$ flies on NF or HFD. Arrows indicate lipid droplets; cardiomyocyte nuclei are stained green by DAPI. See also Figure S1.

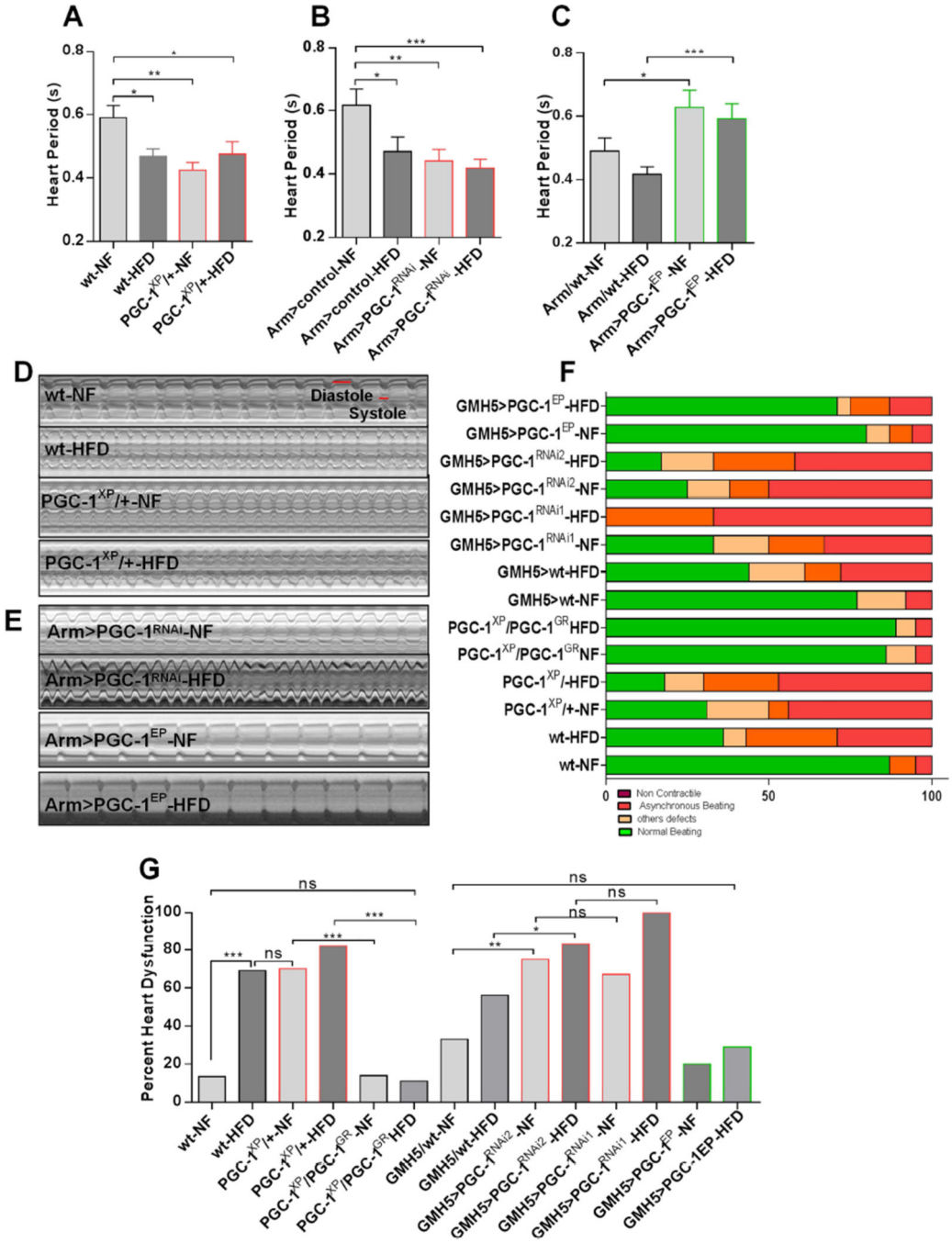

Figure 2. HFD-Induced Cardiac Dysfunction is Modulated by *PGC-1/srl* Function

(A) Heart periods of wt and *PGC-1* heterozygous flies. Results are the mean ± SEM of 45 ≤ n ≥ 33. *P < 0.05, **P < 0.01, one way ANOVA test.

(B) Heart periods of control and *PGC-1* knockdown flies. Results are expressed as the mean ± SEM of 22 ≤ n ≥ 20. *P < 0.05, **P < 0.01, ***P < 0.001, one way ANOVA test.

(C) Heart periods of control and *PGC- 1*-overexpressing flies. Results are the mean ± SEM of 32 ≤ n ≥ 16. *P < 0.05, ***P < 0.001, one way ANOVA test.

(D–E) Representative M-modes traces (5s) showing heart wall movements (Y-axis) versus time (X-axis). Stills are from high-speed movies of semi-intact fly heart preparations for (D) wt and *PGC-1*$^{XP/+}$ flies on NF or HFD, and (E) *PGC-1* knockdown (Arm>*PGC- 1*^{RNA}) and *PGC-1*-overexpressing (Arm>*PGC-1*^{EP}) flies on NF or HFD.

(F) Graphical representation of the proportion of *PGC-1/srl* mutant, RNAi and overexpression flies displaying heart dysfunction phenotypes, classified as non-contractile regions, asynchronous beating, and other defects (dysfunctional ostia, narrowed heart regions, and transmission defects).

(G) Quantification of heart defects shown in (F). Statistical significance was determined using chi-square test $33 \leq n \geq 14$. wt-NF vs. wt- HFD, $x^2 = 15$; $P < 0.001$. wt-HFD vs. *PGC-1*$^{XP/+}$-NF, $x^2 = 0.008$; ns. *PGC-1*$^{XP/+}$-NF vs. *PGC-1*^{XP}/*PGC-1* ^{GR}-NF, $x^2 = 15.78$; $P < 0.001$. *PGC- 1*$^{XP/+}$-HFD vs. *PGC-1*^{XP}/*PGC-1*^{GR}-HFD, $x^2 = 23.00$; $P < 0.001$. wt-NF vs. *PGC- 1*^{XP}/*PGC-1*^{GR}-HFD, $x^2 = 0.002$; ns. GMH5/wt-NF vs. GMH5>*PGC-1*^{RNAi1}-NF, $x^2 = 7.03$; $P < 0.01$. GMH5/wt-HFD vs. GMH5>*PGC-1*^{RNAi2}-HFD, $x^2 = 3.33$; $P < 0.05$. GMH5>*PGC-1*^{RNAi2}-NF vs. GMH5>*PGC-1*^{RNAi1}-NF, $x^2 = 0.17$; ns. GMH5>*PGC-1*^{RNAi2}-HFD vs. GMH5>*PGC-1*^{RNAi1}-HFD, $x^2 = 2.30$; ns. GMH5/wt-NF vs. GMH5>*PGC-1*^{EP}-HFD $x^2 = 013$; ns. not significant (ns). See also Figure S2 and Movies S1–S7.

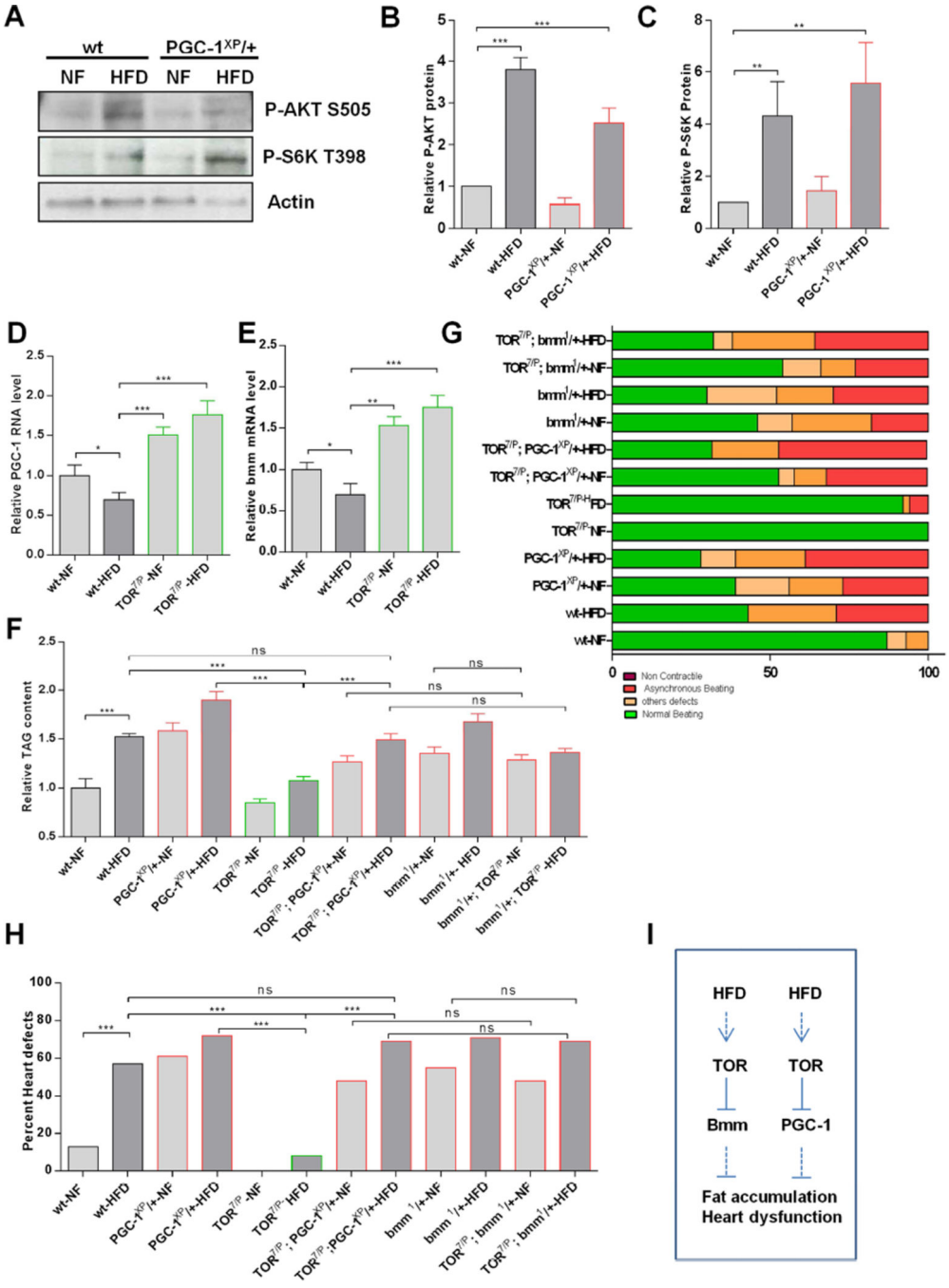

Figure 3. TOR is an Upstream Regulator of *PGC-1/srl* and *bmm* in HFD-Induced Heart Dysfunction

(A) Western blots of phospho-AKT and phospho-S6K from wt and *PGC-1/srl* heterozygous flies on NF and HFD. Actin was used as loading control.

B–C: quantification of relative P (S6K) and P (AKT) protein based on densitometry measurements. Statistics were done using the Kruskal-Wallis test. **P < 0.05, ***P <0.001.

(D–E) Relative *PGC-1/srl* (D) and *bmm* (E) mRNA levels in wt and *TOR* mutant on NF and HFD. Results are expressed as the fold difference compared with wt flies. *P < 0.05, one way ANOVA test.

(F) Relative TAG content in *PGC-1/srl, bmm* and *TOR* single and double mutant flies on NF and HFD. Results are expressed as the fold difference in whole fly TAG compared with wt-NF flies and are the mean ± SEM of $66 \leq n \geq 18$. . **P < 0.01, ***P < 0.001, one way ANOVA test.

(G) Graphical representation of the proportion of *PGC-1/sr, bmm and TOR* single and double mutant flies displaying heart dysfunction phenotypes, classified as non-contractile regions, asynchronous beating, and other defects (dysfunctional ostia, narrowed heart regions, and transmission defects).

(H) Quantification of heart defects shown in (G). Statistical significance was determined using chi-square test $39 \leq n \geq 19$. wt-NF vs. wt-HFD, $x^2 = 12$; P < 0.001. wt-HFD vs. *TOR7/P*-HFD, $x^2 = 19.33$; P <0.001. *PGC-1^{XP}/+*-HFD vs. TOR$^{7/P}$-HFD, $x^2 = 23.58$; P < 0.001. TOR$^{7/P}$-HFD vs. *TOR$^{7/P}$; PGC-1^{XP}/+*-HFD, $x^2 = 23.58$; P < 0.001. wt-HFD vs. *TOR$^{7/P}$; PGC-1^{XP}/+*-HFD, $x^2 = 0.46$; ns. *TOR$^{7/P}$; PGC-1$^{XP/+}$*-NF vs. *TOR$^{7/P}$; bmm^{1}/+-NF*$x^2 = 0$; ns. *TOR$^{7/P}$; PGC-1$^{XP/+}$*-HFD vs. *TOR$^{7/P}$; bmm^{1}/+*-HFD, $x^2 = 0.01$; ns. *Bmm^{1}/+-NF* vs. *TOR$^{7/P}$; bmm^{1}/+-HFD*$x^2 = 106$; ns. . ns: not significant.

(I) Schematic of proposed regulatory network. HFD feeding activates TOR signaling, which reduces *PGC-1/srl* and *bmm* expression to lead to fat accumulation and heart dysfunction. See also Figure S3.

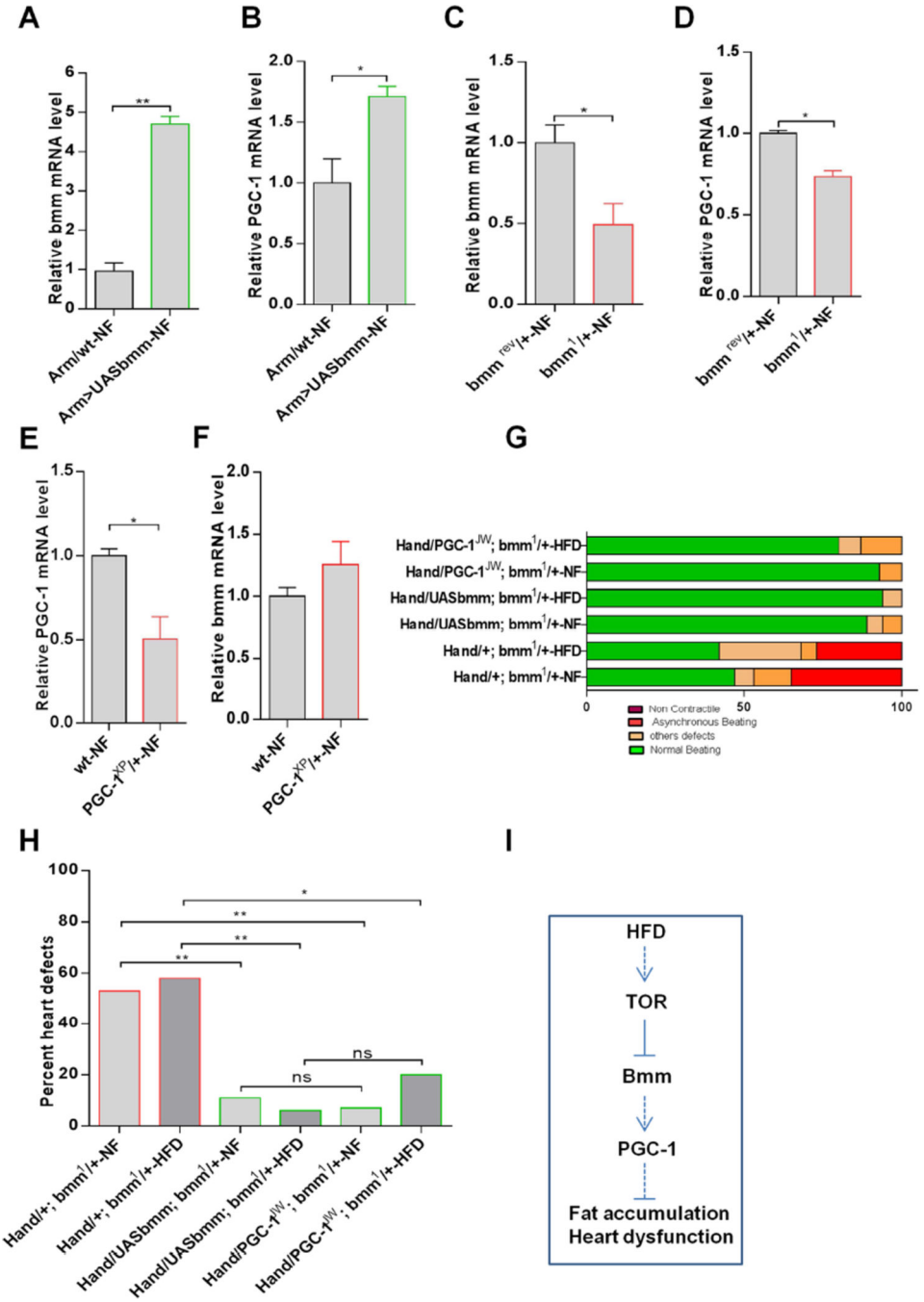

Figure 4. *ATGL/bmm* is an Upstream Regulator of *PGC-1/srl* in HFD-Induced Heart Dysfunction

(A–F): Relative expression of *bmm* (A, C, and F) and *PGC-1/srl* (B, D, and E) mRNA in *bmm* KD /overexpression flies and in *PGC-1/srl* mutant flies. *P < 0.05, **P < 0.01, student t-test.

(G) Graphical representation of the proportion of flies with cardiac-specific manipulation of *bmm* and *PGC-1/srl* in a *bmm* mutant background displaying heart dysfunction phenotypes, classified as non-contractile regions, asynchronous beating, and other defects (dysfunctional ostia, narrowed heart regions, and transmission defects).

(H) Quantification of heart defects shown in (G). Statistical significance was determined using chi-square test $19 \leq n \geq 14$. *Hand/+; bmm^{1}/+-NF* vs. *Hand/UASbmm; bmm^{1}/+-NF*\$^{2}$ = 10.16; $P < 0.01$. *Hand/+; bmm^{1}/+-HFD* vs. *Hand/UASbmm; bmm^{1}/+-HFD*$x^2 = 10.28$; $P < 0.01$. *Hand/+; bmm^{1}/+-NF* vs. *Hand/PGC-1^{JW}; bmm^{1}/+-NF*\$^{2}$ = 7.36; $P < 0.01$. Hand/+; *bmm^{1}/+-HFD* vs. *Hand/PGC-1^{JW}; bmm^{1}/+-HFD*$x^2 = 4.96$; $P < 0.05$. *Hand/UASbmm; bmm^{1}/+-NF* vs. *Hand/PGC-1^{JW}; bmm^{1}/+-NF*$x^2 = 0.05$; ns. *Hand/UASbmm; bmm^{1}/+-HFD* vs. *Hand/PGC-1^{JW}; bmm^{1}/+-HFD*$x^2 = 1.3$; ns.

(I) Schematic of proposed regulatory network. HFD consumption activates TOR signaling, which inhibits the expression of *bmm* and, subsequently, *PGC-1/srl* in the same axis, leading to fat accumulation and heart dysfunction. See also Figure S4.

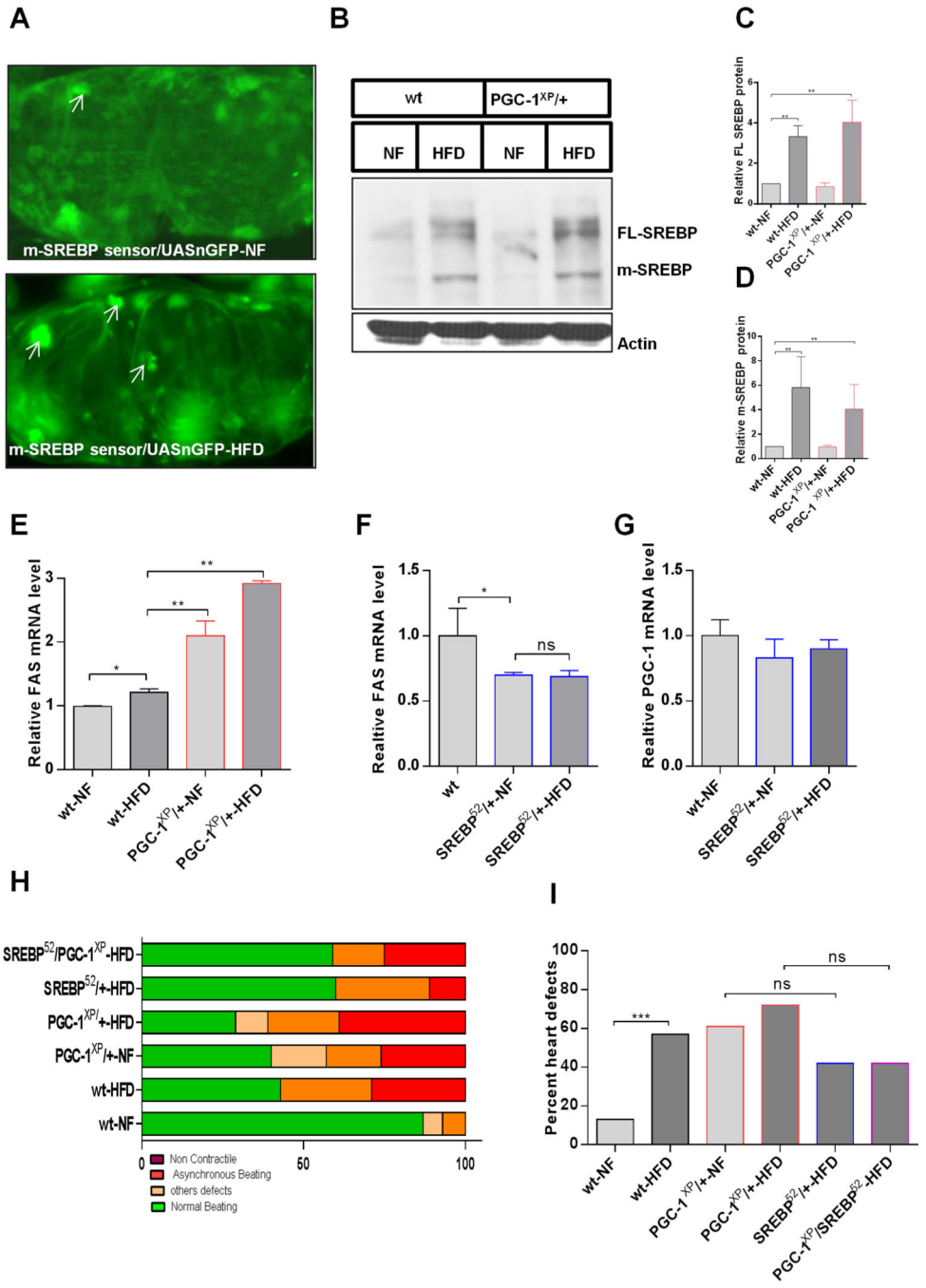

Figure 5. SREBP and *PGC-1/srl* Function through Parallel Pathways in HFD-Induced Heart Dysfunction

(A) Representative micrographs of sections of hearts of flies carrying a UAS-GFP/SREBP sensor and fed NF (top) or HFD (bottom). Sections were stained with anti-GFP antibody (green). Arrowheads indicate increased GFP staining (SREBP activation) in the nuclei of hearts from HFD-fed flies.

(B–D): Western blots of SREBP (FL: full length, m: mature isoform) in whole fly extracts of wt and *PGC-1^{XP}/+* flies on NF or HFD (A). Actin was included as a loading control. C and

D are quantification of relative FL SREBP protein (C) and m-SREBP from westerns in B (D). Kruskal Wallis test was used for the statistics. **P < 0.01.

(E) Relative expression of *FAS* mRNA in wt and *PGC-1*$^{XP/+}$ flies on NF or HFD. Results are mean ± SD of n = 30 *P < 0.05, **P < 0.01, one way ANOVA test.

(F–G) Relative expression of (F) *FAS* and (G) *PGC-1/srl* mRNA in wt and SREBP mutant flies on NF or HFD. Results are mean ± SD of n = 30 *P < 0.05. ns: not significant, one way ANOVA test.

(H) Graphical representation of the proportion of *SREBP* and *PGC-1/srl* mutant flies displaying heart dysfunction phenotypes, classified as non-contractile regions, asynchronous beating, and other defects (dysfunctional ostia, narrowed heart regions, and transmission defects).

(I) Quantification of heart defects shown in (F). Statistical significance was determined using chi-square test, 26 ≤ n ≥ 14. wt-NF vs. wt-HFD, $x^2 = 12$; P < 0.001. *PGC-1*$^{XP/+}$-NF vs. *SREBP*$^{52/+}$- NF, $x^2 = 0.3$; ns. *PGC-1*$^{XP/+}$- HFD vs. *SREBP*$^{52/+}$- HFD, $x^2 = 3$; ns. *SREBP*$^{52/+}$*-NF* vs. *SREBP*^{52}*/PGC-1* ^{XP}-NF, $x^2 = 1.5$; ns. *SREBP*$^{52/+}$*-HFD* vs. *SREBP*^{52}*/PGC-1*^{XP}-HFD, $x^2 = 0.01$; ns: not significant. See also Figure S5.

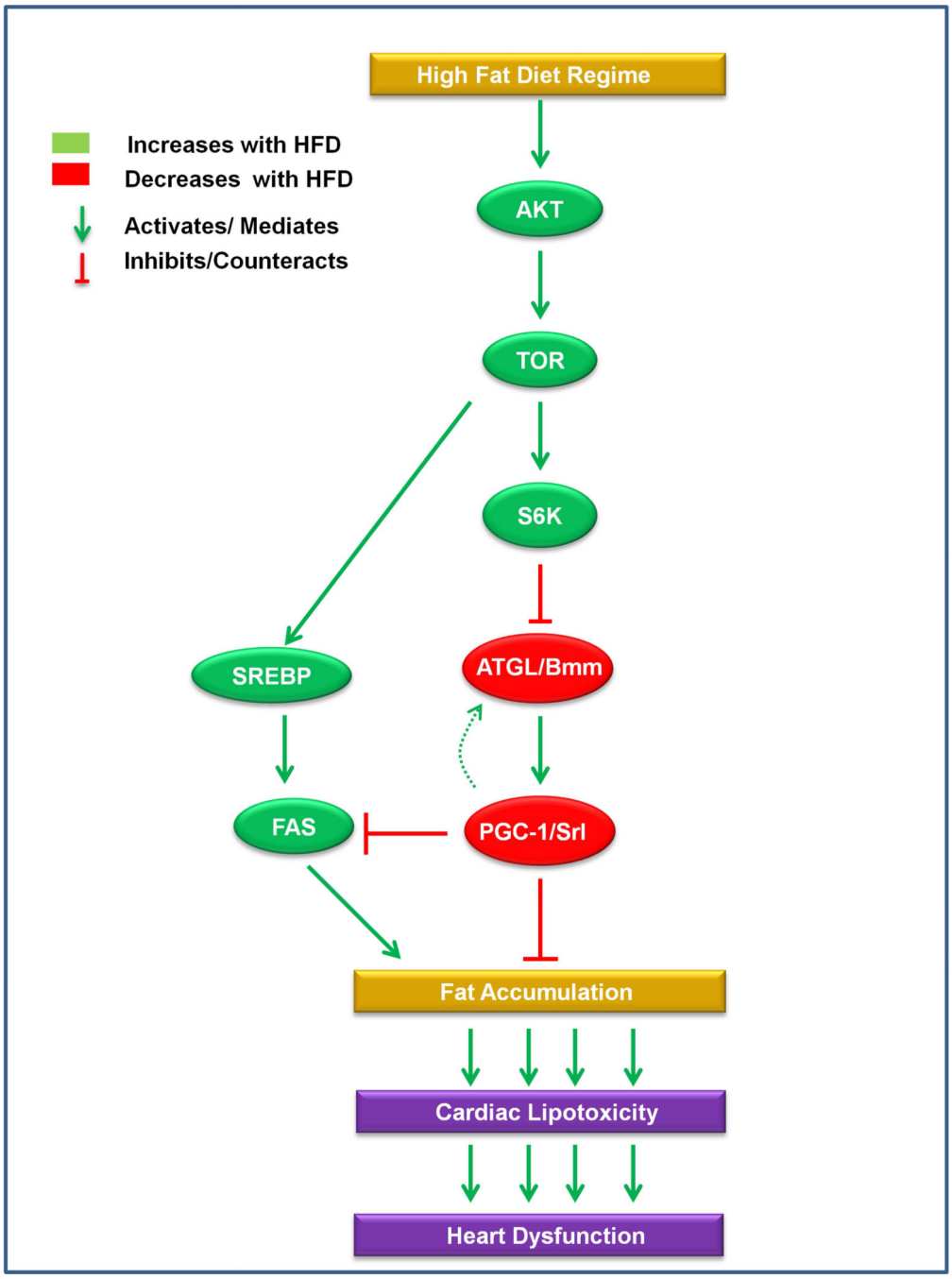

Figure 6. Model of genetic network in HFD-induced cardiac lipotoxicity

HFD feeding causes increased TOR signaling that leads to decreased *PGC-1/srl* expression via decreased *ATGL/bmm* function mediating obesity and cardiac lipotoxicity. There may be some feedback from PGC-1/*srl* to *ATGL*/*bmm* in the heart itself, but in regards to modulation of HFD-dependent heart dysfunction PGC-1/*srl* acts downstream of *ATGL*/*bmm*. SREBP is also activated by TOR and is interconnected to the *ATGL/bmm-PGC-1/srl* axis via *FAS* to contribute to the regulation of HFD-inflicted cardiac lipotoxicity.